# AIR FORMALDEHYDE EXPOSURE AND DPC IN BUCCAL CELLS OF UNDERGRADUATE STUDENTS

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## ABSTRACT

**Objective**: To study the effect of formaldehyde on DNA–protein crosslinks (DPC) in buccal cells in students who were taking anatomy course. **Methods**: The modified KCl-SDS precipitation assay was applied. This method has been used to explore DPC induced by different pollutants in laboratory studies. **Results**: 37 students in medical school and 40 in school of sciences were studied. The frequency of exposure to formaldehyde was 6 h per week in exposed group. Concentrations of formaldehyde in anatomy laboratory ranged from 0.42 to 1.57 mg/m$^3$. Exposure to formaldehyde resulted in an increase of DNA-protein crosslinks. DNA-protein crosslinks in exposed and nonexposed students were 25.72±6.48% and 22.88±5.34% ($p<0.05$), respectively. Air formaldehyde caused a significant increase in DNA–protein crosslinks of the females in two groups (27.72±5.76% and 22.29±4.20%, $p<0.01$). However, there was no difference in males (24.02±6.72 and 23.48±6.33, $p>0.05$). **Conclusions**: The results suggest that exposure to formaldehyde in medical students increase the frequency of DNA–protein crosslinks in buccal cells. Our results also indicate that females may be more sensitive to formaldehyde exposure.

## INDEX TERMS

Epidemiological study, Formaldehyde, DNA–protein crosslinks, Buccal cell

## INTRODUCTION

Formaldehyde is a flavorless, colorless gas with stimulated smell. It dissolves easily and its 35%-40% solution is called formalin. We usually use formalin as corpse antiseptic in cadaver dissection practice laboratory. Some studies show that there is the increase of micronucleus (MN) frequency in blood lymphocyte, nasal mucosa cell and buccal cell in students after exposing to formaldehyde. In 1996 the expert panel of American health foundation reported that: In the genetic toxicity and carcinogenic process of the gas formaldehyde, the most important part is the forming of DNA-protein crosslinks, DPC (Conaway CC et al 1996). Many studies use the DPC of lymph and nasal as the Biomarker for formaldehyde exposure. Actually DPC is target organ cell for formaldehyde exposure (Shaham J et al 1996, Kuykendall JR et al 1995). But right now there is no report about epidemiological DPC. Therefore we collect the samples of DPC from the medical school students just taking the anatomy course. Using the modified KCl-SDS precipitation assay reported by Zhitkovich A. & Costa M.A (1992), we detected the DPC to study how the exposure to formaldehyde affects the DPC.

## RESEARCH METHODS

### Study subjects

A total of 37 medical students (20 boys, 17 girls) who were taking anatomy course served as subjects. They had been exposed to formaldehyde in gross anatomy laboratory. The frequency of exposure to formaldehyde was 6 h per week in exposed group. We also included an unexposed group (20 boys, 20 girls) that had not experienced formaldehyde exposure. The unexposed group was the students of school of sciences. Both groups were freshmen of university.

### Measurement of formaldehyde concentration in air

This study was conducted in a gross anatomy laboratory at a medical school. The laboratory dimensions were 9.42m×8.93m× 2.93m. There was one door. The door was closed when not in use. There were no windows. During the study there were 8 dissecting tables in use. The dissecting tables were spread evenly along the length of the laboratory in two rows and 4 tables in every row. Six supply diffusers and 10 air return grills provided general ventilation to the laboratory. The diffusers were uniformly spaced on the ceiling along the centerline axis of the room. Eight of the air returns were at floor level. There was recirculation of air removed from the laboratory via the returns. 3 sampled points were chosen. Point A lay beside the 4th dissecting table in right row. Point B lay in the

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center of the laboratory and point C was beside the 1st table on the left. The concentration of formaldehyde was measured with the Model Interscan4160-2 formaldehyde analysis instrument of U.S.A. Temperature and relative humidity were measured in order to ensure that the acceptable parameters of the sampling instrument were not exceeded. The classroom of medical school and dormitory of two schools were measured.

**DPC assay**

Buccal cell sample experimenter branch water gargle 3 times, rinses the food residue of oral cavity well at first. Medium-sized dynamics brush both sides oral cavity mucous membrane the wall is each 30 times from head to foot with fur toothbrush, the range tries one's best to be extensive. Gargle with the physiological saline of 10ml after finishing brushing; let the physiological saline wash the mucous membrane brushed. Collect the gargle physiological saline with a tube.

Buccal cell samples were lysed in a 0.5% SDS solution as previously described. The samples were frozen at -70°C and the DNA-protein crosslinks induced by all the agents examined were stable when stored under these conditions. Cell samples were thawed at 37°C and the DNA was sheared by passing the cell lysates 10 times through a 1 ml pipet tip. The lysates were expelled into a tube by applying medium pressure but extensive foaming is always avoided. 100μl of 1M KCI, 100 mM Tris (pH 7.5), was then added. The content was mixed by vortexing the tube for 5 s at maximal speed and the tubes were then heated for 10 min at 65°C. Samples were removed from the water bath, inverted 3 times and then placed on ice for 5 min to form potassium dodecyl sulfate precipitates. The precipitate was collected by centrifugation at 11000rpm for 5 min at 4°C. The supernatant is removed and the pellet resuspended in 100 mM of KCl, 20 mM Tris-HCl (pH7.5) by brief vortexing at the highest setting. The samples were again heated at 65°C for 10 min and the washing steps described above, as well as the heating steps, were repeated 2 more times. Protein-linked DNA was released from the final K-SDS precipitate by treatment with 0.2 mg/ml proteinase K in 0.5 ml solution containing 100 mM KCI, 20mM Tris-HCl (pH 7.5). DNA was detected using Hoechst 33258. DNA standards were prepared at concentrations of 100, 200, 300,400, 500, 750, 1000, 2000, 3000ng/ml, 1 ml of a standard DNA or the entire supernatant from the potassium dodecyl sulfate pellet was taken and mixed with 1 ml of freshly prepared Hoechst dye reagent, 200 ng/ml. Precaution was taken to avoid exposure to light at this point, and the samples were placed in the dark for 30 min. Fluorescence was assessed by exitation at 350 nm and the emitted light was measured at 450nm. The following is the formula for calculating DPC percentage. Where C is the amount of combined DNA and U is amount of uncombined DNA.

$$DPC(\%) = \frac{C}{C+U} \times 100\% \quad (1)$$

**Statistical analyses**

The mean, standard deviation and standard error were calculated for each biomarker. The significance of the differences between exposed and non-exposed endpoint means were analyzed using the Student's t-test. Significance levels of 5% or less were considered to be significant. All p values were two-tailed.

**RESULTS**

**General characteristics**

The general characteristics of the study population are summarized in table 1.The mean ages of exposed groups was not significantly different from that of the control groups ($p > 0.05$).

***Table 1.*** *General Characteristics of Subjects*

| Group | N | Age | Sex（M/F） |
|---|---|---|---|
| Exposed | 37 | 19.24±1.09 | 20/17 |
| Nonexposed | 40 | 19.55±0.99 | 20/20 |

**Formaldehyde Concentration**

Table 2 demonstrates the concentrations in the gross anatomy laboratory. All data of formaldehyde concentration in our study was higher than the threshold limit value (TLV) for formaldehyde (0.08 $mg/m^3$). Before we opened the cover of dissecting tables, average concentrations of three sampled point was 0.42 $mg/m^3$. The concentrations sharply rose to the peak 12.04 $mg/m^3$ after the cover had been opened. Run the ventilation facilities at the 37min, then formaldehyde went back rapidly and tended toward stability at the time from 50th to 60th, the lowest concentrations of three sampled points were average 1.57$mg/m^3$. Half time of anatomy course was about theory

study and did not need open the cover. The cover was opened only when the practice study in the other half time. So the formaldehyde exposure level of medical students was between 0.42 mg/m$^3$ to 1.57 mg/m$^3$. Formaldehyde concentrations in school of sciences classroom and two groups of dormitories were shown in table 3. Formaldehyde concentrations in the three rooms were not different and lower than 0.08 mg/m$^3$.

***Table 2.*** *Formaldehyde concentration（mg/m$^3$）*

| Room | Time (min) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0th | 10th | 20th | 30th | 40th | 50th | 60th |
| A | 0.44 | 4.89 | 8.01 | 9.35 | 2.85 | 1.9 | 1.45 |
| B | 0.32 | 7.83 | 12.04 | 9.81 | 2.63 | 2.01 | 1.56 |
| C | 0.49 | 8.59 | 10.34 | 11.18 | 2.21 | 1.69 | 2.21 |

Note: 1. at the time of 0th the cover of dissecting tables wasn't opened. 2. Opened the cover at the 10min. 3. Run the ventilation facilities at the 37min.

***Table3.*** *Formaldehyde concentration in the dormitory and classroom in the school of sciences and medical school*

| Places | Formaldehyde (mg/m$^3$) | | |
|---|---|---|---|
| Science school classroom | 0.012 | 0.024 | 0.024 |
| Science school dormitory | 0.012 | 0.024 | 0.037 |
| Medical school dormitory | 0.037 | 0.037 | 0.024 |

**DPC results**

Two groups of student's buccal cell DPC percentage were 25.72± 6.48% and 22.88± 5.34% respectively. The data indicated that there was a significance difference between two groups, P <0.05 (Table 4). Comparing according to the sex, DPC levels of female exposed group was higher than non-exposed, but there was not a difference between the two groups of male students.

***Table 4.*** *Buccal cell DPC percentage of students（$\overline{X}\pm S$）*

| Group | Male | | Female | | Total | |
|---|---|---|---|---|---|---|
| | N | DPC% | N | DPC% | N | DPC% |
| Exposed | 20 | 24.02±6.72 | 17 | 27.72±5.76 | 37 | 25.72±6.48 |
| Nonexposed | 20 | 23.48±6.33 | 20 | 22.29±4.20 | 40 | 22.88±5.34 |
| t | | 0.26 | | 3.31 | | 2.1 |
| p | | 0.7953 | | 0.0022 | | 0.0391 |

**DISCUSSIONS**

Recent medical researches show that many external compounds can cause DPC. Though different compounds cause DPC in different ways, once DPC comes into being, it of course has great effect on the function and structure of the DNA. Possibly it has some relationship with crossing nucleoprotein because nucleoprotein is one of the important components keeping the DNA structure and participating in controlling the copy and record. Furthermore, the DPC is very difficult to be repaired, so in the process of coping DNA, some important DNA will loose. In a word, it makes sense to choose DPC as a biomarker for formaldehyde exposure.

To date, four studies have evaluated the cytogenetic alterations in the epithelial buccal mucosa cells from individuals exposed to various levels of FA. Norppa et al. (1993) reported an increased MN frequency in the buccal cells of workers in a plywood plant and fibre glass factory, while no change was observed in lymphocytes. Suruda et al. (1993) demonstrated that low level exposure to formaldehyde (0.33 ppm.) was associated with cytogenetic changes in the epithelial cells of the buccal cavity and in the blood lymphocytes of male students participating in an embalming course. Titenko-Holland et al. (1996) found a 3.3-fold increase in MN frequency in buccal cells from mortuary science students exposed to FA, whereas in nasal cells there was none. Ying et al. (1997) showed significant increases (1.5-fold) in the MN frequencies in the buccal and nasal cells but not in the lymphocytes of

anatomy class students exposed to FA (0.5 ppm.). Further, an increased MN frequency was recently found in these subjects in exfoliated nasal cells (Burgaz et al. 2001).

The research reports above show that when exposed to low concentration formaldehyde, buccal cell has obvious change of cytotoxicity. However we usually choose blood lymphocyte and nasal mucosa cell as the targets in the study of DPC exposure to formaldehyde. Recently some domestic scholars set up a model of buccal cell DPC exposure to formaldehyde. Anyway there are some virtues using buccal cell to test DPC: (1) High sensitivity in exposure to low concentration formaldehyde. (2) Convenient sampling: don't need complicate equipment and special reagent; the procedure is simple, painless and with high acceptance which is helpful to sampling from a great amount of people. 3. It is easy to store the samples without any change.

Shaham et al. (1996) measured the formation of DNA-protein cross links in peripheral white blood cells of occupationally exposed workers (n=12) and unexposed controls (n=8). The average length of occupational exposure was 13 years. Venous blood samples were collected from each worker and were processed to isolate DNA-protein cross links. Personal monitoring devices indicated formaldehyde concentrations of 2.8–3.1 ppm during peak work and an average concentration of 1.46 ppm at times when work was usually completed. Exposure to formaldehyde resulted in a significant increase in the incidence of DNA-protein crosslinks. (Mean±sd) incidences in exposed and non-exposed workers were 28±6 and 22±6%, respectively. His DPC of non-exposed workers is something like the DPC in this report, owever his DPC of exposed worker is higher. The difference possibly comes from the different number of samples and exposure time. Few of the reports have mentioned the effect of sex in exposure to formaldehyde. This report gives the result of increasing of buccal cell's DPC because of touching the formaldehyde in anatomy course. We find that there is a great difference between female exposed and non-exposed students, but none for male, also no difference between female and male exposed students. Because student exposed to formaldehyde at a low level and for a relatively short time, it seems that female is more sensitive to formaldehyde than male. The differences above come from some factors: (1) Speed of excretive of the foreign source chemical compound; Animal experiment indicate general male excretive speed of chemical compound faster than female. (2) Gender differences eliminated. (3) Influence of hormone and inherent cause; The difference of levels of hormone may influence the sensitiveness of the foreign source chemical compound in adolescence.

There are good ventilation facilities in the dissection laboratory of this research, but its air formaldehyde is density higher than the threshold limit value (TLV) for formaldehyde (0.08 mg/m3). So it is important to pay special attention to the personal protection of medical students who are taking anatomy course.

## CONCLUSIONS

The results suggest that exposure to formaldehyde in medical students increase the frequency of DNA–protein crosslinks in buccal cells. Our results also indicate that females may be more sensitive to formaldehyde exposure.

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